

AMENDMENTS TO THE CLAIMS

1. (Cancelled) A method for cloning a nucleic acid fragment into a vector by flanking the fragment with first and second adapter sequences, and contacting the fragment with the vector having sequences homologous to the first and second adapter sequences under conditions such that the nucleic acid fragment is incorporated into the vector by homologous recombination *in vivo* in a host cell.

2. (Cancelled) The method of Claim 1, wherein the nucleic acid fragment is generated by polymerase chain reaction (PCR).

3. (Cancelled) The method of Claim 1, wherein the first and second adapter sequences are incorporated to the nucleic acid fragment by PCR.

4. (Cancelled) The method of Claim 3, wherein the resulting nucleic acid fragment is a transcriptionally active PCR fragment.

5. (Cancelled) The method of Claim 1, wherein said first and second adapter sequences further comprise a functional element.

6. (Cancelled) The method of Claim 5, wherein the functional element is selected from the group consisting of a promoter, a terminator, a nucleic acid fragment encoding a selection marker gene, a nucleic acid fragment encoding a protein, encoding a fusion tag, a nucleic acid fragment encoding a portion of a selection marker gene, a nucleic acid fragment encoding a growth promoting protein, a nucleic acid fragment encoding a transcription factor, and a nucleic acid fragment encoding an autofluorescent protein (e.g. GFP), a nucleic acid fragment encoding a peptide.

7. (Cancelled) The method of Claim 1, wherein the nucleic acid fragment comprises an additional element.

8. (Cancelled) The method of Claim 7, wherein the additional element is selected from the group consisting of an operably linked promoter, a termination sequence, an operon, a fusion tag, a signal peptide for intracellular or intercellular trafficking, a peptide, a protein, an antisense sequence, a ribozyme, and a protein binding site.

9. (Cancelled) The method of Claim 6, wherein the promoter is selected from the group consisting of a promoter from a plant, a plant pathogen, a mammal or a mammalian pathogen, a fungus, a bacterium or a bacterial phage.

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10. (Cancelled) The method of Claim 6, wherein the terminator sequence is derived from a plant, a procaryotic source and a eukaryotic source.
11. (Cancelled) The method of Claim 6, wherein, the operon is selected from the group consisting of lac operon, Tet/on operon, Tet/off operon, and trp operon.
12. (Cancelled) The method of Claim 6, wherein the fusion tag is selected from the group consisting of 6x or 8x his-tag, GST tag, fluorescent protein tag, Flag tag, and HA tag.
13. (Cancelled) The method of Claim 8, wherein the protein comprises enzymes, receptors, transcription factors, lymphokines, hormones, and antigens.
14. (Cancelled) The method of Claim 1, wherein the vector comprises a plasmid, a cosmid, and a bacterial artificial chromosome (BAC).
15. (Cancelled) The method of Claim 13, wherein the plasmid is selected from the group consisting of CoE1, PR100, R2, and pACYC.
16. (Cancelled) The method of Claim 13, wherein the plasmid comprises a functional selection marker.
17. (Cancelled) The method of Claim 16, wherein the functional selection marker is selected from kanamycin resistance gene, kenamycin resistance gene; ampicillin resistance gene, blasticidin resistance gene, carbonicillin resistance gene, tetracycline resistance gene, and chloramphenicol resistance gene.
18. (Cancelled) The method of Claim 1, wherein the vector comprises a dysfunctional selection marker that lacks a critical element, and wherein the critical element is supplied by said nucleic acid fragment upon successful homologous recombination.
19. (Cancelled) The method of Claim 18, wherein the dysfunctional selection marker is selected from kanamycin resistance gene, ampicillin resistance gene, blasticidin resistance gene, carbonicillin resistance gene, tetracycline resistance gene, and chloramphenicol resistance gene
20. (Cancelled) The method of Claim 18, wherein the dysfunctional selection marker is a reporter gene.
21. (Cancelled) The method of Claim 20, wherein the reporter gene is lacZ.

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22. (Cancelled) The method of Claim 1, wherein the vector comprises a negative selection element detrimental to host cell growth, and wherein the negative selection element is disabled by said nucleic acid fragment upon successful homologous recombination.

23. (Cancelled) The method of Claim 22, wherein the negative selection element is inducible.

24. (Cancelled) The method of Claim 22, wherein the negative selection element is a mouse GATA-1 gene.

25. (Cancelled) The method of Claim 1, wherein the vector comprises a dysfunctional selection marker and a negative selection element.

26. (Cancelled) The method of Claim 1, wherein the host cell is a bacterium.

27. (Cancelled) The method of Claim 24, wherein the bacterium is capable of *in vivo* recombination.

28. (Cancelled) The method of Claim 25, wherein the bacterium is selected from the group consisting of JC8679, TB1, DH5 α , DH5, HB101, JM101, JM109, and LE392.

29. (Cancelled) The method of Claim 16, wherein the plasmid is maintained in the host cell under the selection condition selecting for the functional selection marker.

30. (Cancelled) The method of Claim 1, wherein said first and second adapter sequences are at least 11 bp.

31. (Cancelled) The method of Claim 1, wherein said first and second adapter sequences are at least 40 bp.

32. (Cancelled) The method of Claim 1, wherein said first and second adapter sequences are at least 50 bp.

33. (Cancelled) The method of Claim 1, wherein said first and second adapter sequences are at least 60 bp.

34. (Cancelled) The method of Claim 1, wherein said first and second adapter sequences are greater than 60 bp.

35. (Cancelled) The method of Claim 1, wherein the contacting comprises transforming a host cell with the vector and the nucleic acid fragment.

36. (Cancelled) The method of Claim 29, wherein the transformation comprises electroporation or chemical transformation.

37. (Cancelled) The method of Claim 1, wherein the host cell comprises a cell bearing the vector.

38. (Cancelled) The method of Claim 37, wherein the cell is a bacterium.

39. (Cancelled) The method of Claim 38, wherein the bacterium is capable of *in vivo* recombination.

40. (Cancelled) The method of Claim 39, wherein the bacterium is selected from the group consisting of JC8679, TB1, DH5 α , DH5, HB101, JM101, JM109, LE392.

41. (Cancelled) The method of Claim 37, wherein the contacting comprises transforming the host cell bearing the vector with the nucleic acid fragment.

42. (Cancelled) The method of Claim 37, wherein the vector is a plasmid.

43. (Cancelled) The method of Claim 42, wherein the plasmid comprises a functional selection marker.

44. (Cancelled) The method of Claim 43, wherein the functional selection marker is a resistance gene selected from kanamycin, kenamycin, ampicillin, blasticidin, carbonicillin, tetracycline, and chloramphenicol.

45. (Cancelled) The method of Claim 42, wherein the plasmid comprises a dysfunctional selection marker that lacks a critical element, and wherein the critical element is supplied by said nucleic acid fragment upon successful homologous recombination.

46. (Cancelled) The method of Claim 45, wherein the dysfunctional selection marker is selected from kanamycin resistance gene, kenamycin resistance gene, ampicillin resistance gene, blasticidin resistance gene, carbonicillin resistance gene, tetracycline resistance gene, and chloramphenicol resistance gene.

47. (Cancelled) The method of Claim 45, wherein the dysfunctional selection marker is a reporter gene.

48. (Cancelled) The method of Claim 47, wherein the reporter gene is lacZ.

49. (Cancelled) The method of Claim 37, wherein the vector comprises a negative selection element detrimental to host cell growth, and wherein the negative selection element is disabled by said nucleic acid fragment upon successful homologous recombination.

50. (Cancelled) The method of Claim 49, wherein the negative selection element is inducible.

51. (Cancelled) The method of Claim 50, wherein the negative selection element is GATA-1 gene.

52. (Cancelled) The method of Claim 37, wherein the vector comprises a dysfunctional selection marker and a negative selection element.

53. (Currently Amended) A method for cloning a nucleic acid insert into a vector comprising:

providing a nucleic acid insert that comprises ~~flanked by~~ first and second ~~adapter~~ flanking homology sequences, and that is adapted for recombining with homologous sequences in a vector, and wherein the vector has a dysfunctional selection marker lacking a critical element and said nucleic acid insert contains said critical element;

contacting the nucleic acid insert with the vector to effect recombination at homologous sites such that the said critical element is supplied to the vector by the nucleic acid insert and said dysfunctional selection marker is restored to a functional one; and,

selecting the successfully restored selection marker based upon growth of a host containing the successfully recombined vector that allows the host to grow or be identified in a selective environment.

54. (Original) The method of Claim 53, wherein the recombining is by homologous recombination.

55. (Currently Amended) A method for cloning a nucleic acid insert into a vector comprising:

providing a nucleic acid insert that comprises ~~flanked by~~ first and second ~~adapter~~ flanking homology sequences, and that is adapted for recombining with homologous sequences in a vector, and wherein the vector includes a negative selection element detrimental to cell growth;

contacting the nucleic acid insert with the vector to effect recombination at homologous sites such that said negative selection element is disabled; and,

selecting for successful transformation based on the absence of a functional negative selection element.

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56. (Original) The method of Claim 55, wherein the negative selection element is inducible.

57. (Original) The method of Claim 55, wherein the selection step comprises inducing the negative selection element.

58. (Original) The method of Claim 55, further comprising the method of Claim 53.

59. (Original) The method of Claim 55, wherein the negative selection element is disabled by insertion of a sequence encoding a selection marker.

60. (Currently Amended) A system for cloning a nucleic acid fragment into a vector without restriction enzyme, ligase, gyrase, or single stranded DNA binding protein, the system comprising a nucleic acid fragment flanked by first and second ~~adapter~~ flanking sequences that respectively comprise first and second homology sequences, and a vector having sequences homologous to the first and second ~~adapter~~ homology sequences, wherein the nucleic acid fragment is adapted to incorporate into the vector by homologous recombination.

61. (Currently Amended) The system of Claim 60, wherein the nucleic acid fragment flanked by the first and the second ~~adapter~~ flanking sequences that respectively comprise first and second homology sequences is generated by PCR without the use of a restriction enzyme, a ligase, a gyrase, a single stranded DNA binding protein, or any other DNA modifying enzyme.

62. (Currently Amended) The system of Claim 60, wherein the nucleic acid fragment flanked by the first and the second ~~adapter~~ flanking sequences that respectively comprise first and second homology sequences is a transcriptionally active PCR fragment.

63. (Currently Amended) A system for cloning a nucleic acid fragment into a bacterium without the use of a restriction enzyme, a ligase, a gyrase, or a single stranded DNA binding protein, the system comprising a nucleic acid fragment flanked by first and the second ~~adapter~~ flanking sequences that respectively comprise first and second homology sequences, and a bacterium bearing a vector, the vector having sequences homologous to the first and second ~~adapter~~ homology sequences, wherein the nucleic acid fragment is adapted to incorporate into the vector within the bacterium by homologous recombination.

64. (Currently Amended) A kit for cloning a nucleic acid fragment into a vector comprising reagents for amplification of the nucleic acid fragment, wherein the reagents upon

amplification provide for a nucleic acid fragment flanked by first and the second ~~adapter~~ flanking sequences that respectively comprise first and second homology sequences, a linear vector, a competent cell, or a competent cell bearing the linear vector, and the competent cell is ready to be transformed by electroporation or chemical transformation.

65. (Original) The kit of Claim 64, wherein the competent cell or the competent cell bearing the vector is a bacterium.

66. (Original) The kit of Claim 65, wherein the bacterium is capable of *in vivo* recombination.

67. (New) A method for cloning a nucleic acid fragment into a linear vector comprising:

flanking the nucleic acid fragment with first and second flanking sequences respectively comprising first and second homology sequences, wherein said flanking sequences are incorporated onto the nucleic acid fragment by PCR, and wherein said first and second homology sequences are homologous to sequences on the linear vector; and

contacting the nucleic acid fragment with the linear vector under conditions such that the nucleic acid fragment is incorporated into the vector by homologous recombination *in vivo* in a bacterial host cell.

68. (New) The method of Claim 67, wherein the nucleic acid fragment is generated by polymerase chain reaction (PCR).

69. (New) The method of Claim 68, wherein the nucleic acid fragment comprising said first and second flanking sequences is a transcriptionally active PCR fragment.

70. (New) The method of Claim 67, wherein said first and second flanking sequences further comprise a functional element, wherein said functional element is incorporated onto said nucleic acid fragment by PCR.

71. (New) The method of Claim 70, wherein the functional element is selected from the group consisting of a promoter, a terminator, a nucleic acid fragment encoding a selection marker gene, a nucleic acid fragment encoding a protein, encoding a fusion tag, a nucleic acid fragment encoding a portion of a selection marker gene, a nucleic acid fragment encoding a growth promoting protein, a nucleic acid fragment encoding a transcription factor, and a nucleic

acid fragment encoding an autofluorescent protein (e.g. GFP), a nucleic acid fragment encoding a peptide.

72. (New) The method of Claim 67, wherein the nucleic acid fragment comprises an additional element selected from the group consisting of an operably linked promoter, a termination sequence, an operon, a fusion tag, a signal peptide for intracellular or intercellular trafficking, a peptide, a protein, an antisense sequence, a ribozyme, and a protein binding site.

73. (New) The method of Claim 71, wherein the promoter is selected from the group consisting of a promoter from a plant, a plant pathogen, a mammal or a mammalian pathogen, a fungus, a bacterium or a bacterial phage.

74. (New) The method of Claim 71, wherein the terminator sequence is derived from a plant, a procaryotic source and a eukaryotic source.

75. (New) The method of Claim 71, wherein, the operon is selected from the group consisting of lac operon, Tet/on operon, Tet/off operon, and trp operon.

76. (New) The method of Claim 71, wherein the fusion tag is selected from the group consisting of 6x or 8x his-tag, GST tag, fluorescent protein tag, Flag tag, and HA tag.

77. (New) The method of Claim 72, wherein the protein comprises enzymes, receptors, transcription factors, lymphokines, hormones, and antigens.

78. (New) The method of Claim 67, wherein the vector is selected from the group consisting of a plasmid, a cosmid, and a bacterial artificial chromosome (BAC).

79. (New) The method of Claim 78, wherein the plasmid is selected from the group consisting of CoE1, PR100, R2, and pACYC.

80. (New) The method of Claim 78, wherein the plasmid comprises a functional selection marker.

81. (New) The method of Claim 80, wherein the functional selection marker is selected from kanamycin resistance gene, kenamycin resistance gene; ampicillin resistance gene, blasticidin resistance gene, carbonicillin resistance gene, tetracycline resistance gene, and chloramphenicol resistance gene.

82. (New) The method of Claim 67, wherein the vector comprises a dysfunctional selection marker that lacks a critical element, and wherein the critical element is supplied by said nucleic acid fragment upon successful homologous recombination.

83. (New) The method of Claim 82, wherein the dysfunctional selection marker is selected from kanamycin resistance gene, ampicillin resistance gene, blasticidin resistance gene, carbonicillin resistance gene, tetracycline resistance gene, and chloramphenicol resistance gene

84. (New) The method of Claim 82, wherein the dysfunctional selection marker is a reporter gene.

85. (New) The method of Claim 84, wherein the reporter gene is lacZ.

86. (New) The method of Claim 67, wherein the vector comprises a negative selection element detrimental to host cell growth, and wherein the negative selection element is disabled by said nucleic acid fragment upon successful homologous recombination.

87. (New) The method of Claim 86, wherein the negative selection element is inducible.

88. (New) The method of Claim 86, wherein the negative selection element is a mouse GATA-1 gene.

89. (New) The method of Claim 67, wherein the vector comprises a dysfunctional selection marker and a negative selection element.

90. (New) The method of Claim 67, wherein the bacterium is capable of *in vivo* recombination.

91. (New) The method of Claim 89, wherein the bacterium is selected from the group consisting of JC8679, TB1, DH5 α , DH5, HB101, JM101, JM109, and LE392.

92. (New) The method of Claim 80, wherein the plasmid is maintained in the host cell under the selection condition selecting for the functional selection marker.

93. (New) The method of Claim 67, wherein said first and second flanking sequences are at least 11 bp.

94. (New) The method of Claim 67, wherein said first and second flanking sequences are at least 40 bp.

95. (New) The method of Claim 67, wherein said first and second flanking sequences are at least 50 bp.

96. (New) The method of Claim 67, wherein said first and second flanking sequences are at least 60 bp.

97. (New) The method of Claim 67, wherein said first and second flanking sequences are greater than 60 bp.

98. (New) The method of Claim 67, wherein the contacting comprises transforming the host cell with the vector and the nucleic acid fragment.

99. (New) The method of Claim 98, wherein the transformation comprises electroporation or chemical transformation.

100. (New) The method of Claim 67, wherein the bacterial host cell comprises a bacterium bearing the vector.

101. (New) The method of Claim 100, wherein the bacterium is capable of *in vivo* recombination.

102. (New) The method of Claim 100, wherein the bacterium is selected from the group consisting of JC8679, TB1, DH5 α , DH5, HB101, JM101, JM109, LE392.

103. (New) The method of Claim 99, wherein the contacting comprises transforming the bacterial host cell bearing the vector with the nucleic acid fragment.

104. (New) The method of Claim 99, wherein the vector is a plasmid.

105. (New) The method of Claim 104, wherein the plasmid comprises a functional selection marker.

106. (New) The method of Claim 105, wherein the functional selection marker is a resistance gene selected from the group consisting of kanamycin, kenamycin, ampicillin, blasticidin, carbonicillin, tetracycline, and chloramphenicol.

107. (New) The method of Claim 104, wherein the plasmid comprises a dysfunctional selection marker that lacks a critical element, and wherein the critical element is supplied by said nucleic acid fragment upon successful homologous recombination.

108. (New) The method of Claim 107, wherein the dysfunctional selection marker is selected from kanamycin resistance gene, kenamycin resistance gene, ampicillin resistance gene, blasticidin resistance gene, carbonicillin resistance gene, tetracycline resistance gene, and chloramphenicol resistance gene.

109. (New) The method of Claim 107, wherein the dysfunctional selection marker is a reporter gene.

110. (New) The method of Claim 109, wherein the reporter gene is lacZ.

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111. (New) The method of Claim 98, wherein the vector comprises a negative selection element detrimental to host cell growth, and wherein the negative selection element is disabled by said nucleic acid fragment upon successful homologous recombination.

112. (New) The method of Claim 111, wherein the negative selection element is inducible.

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113. (New) The method of Claim 112, wherein the negative selection element is GATA-1 gene.

114. (New) The method of Claim 98, wherein the vector comprises a dysfunctional selection marker and a negative selection element.